PHARMACEUTICAL COMPOSITION CONTAINING DECOY AND USE OF THE SAME

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BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION:

The present invention relates to a composition comprising a compound (e.g., a nucleic acid and a homolog thereof) which specifically binds to a site on a chromosome, to which site a transcriptional regulatory factor binds, and a method of using the same. More particularly, the present invention relates to a composition comprising a decoy compound and a method of using the same.

2. DESCRIPTION OF THE RELATED ART:

A variety of diseases including asthma, cancers, heart diseases, aneurysms, autoimmune diseases, and viral infections manifest varying symptoms and signs and yet it has been suggested that an abnormal expression (an overexpression or underexpression) of one or a few proteins is a major etiologic factor in many cases. In general, the expression of those proteins is controlled by a variety of transcriptional regulatory factors such as transcription activating factors and transcription suppressing genes.

A representative transcriptional factor NF-κB is a transcriptional regulatory factor consisting of heterodimers p65 and p50. NF-κB is typically localized in the cytoplasm where NF-κB is bound by its inhibitory factor Iκ so that intranuclear movement of NF-κB is prevented. However, when a stimulus, such as cytokine, ischemia, reperfusion, or the like, is applied due to any cause, IκB is degraded by phosphorylation. As a result, NF-κB is activated and transferred into the nucleus. In the nucleus, NF-κB binds to an NF-κB binding site on a chromosome and promotes the transcription of a gene downstream thereof.

As genes located downstream of the NF-κB binding site, for example, inflammatory cytokines (e.g., IL-1, IL-6, IL-8, tumor necrosis factor α (TNF α), etc.) and adhesion molecules (e.g., (e.g., VCAM-1, ICAM-1, etc.) are known.

NF-κB may be involved in the onset of progression of tumor malignancy (Rayet B et al., Oncogene 1999 Nov 22; 18(49)6938-47); NF-κB is involved in response of tumor cells to hypoxia stress (Royds JA et al., Mol Pathol 1998 Apr; 51(2):55-61); NF-κB inhibits expression of cytokines and adhesion molecules in synovial membrane cells derived from chronic rheumatoid arthritis patients (Tomita T et al., Rheumatology (Oxford) 2000 Jul; 39(7):749-57); suppression of coordination between a plurality of transcriptional factors including NF-κB changes the malignant phenotypes of various tumors (Denhardt D. T., Crit. Rev. Oncog., 1996; 7(3-4):261-91); downregulation of NF-κB activation due to green tea polyphenol blocks induction of nitric oxide synthesizing enzyme, and suppresses A431 human epidermoid carcinoma cells (Lin J. K., et al., Biochem. Pharmacol., 1999, Sep 15; 58(6):911-5); amyloid β peptide observed in the brains of Alzheimer's disease patients binds to 75-kD neurotrophic receptor ($p75^{NTR}$) in neuroblastoma cells to activate NF-κB in a time-dependent manner and a dose-dependent manner (Kuper P, et al., J. Neurosci. Res., 1998, Dec 15; 54(6):798-804); TNF-α, which is activated by NF-κB, plays an important role in the onset of glomerulonephritis (Ardaillou et al., Bull. Acad. Natl. Med., 1995, Jan; 179(1)103-15); NF-κB decoy *in vivo* blocks expression of cytokines and adhesion molecules in mouse nephritis induced by TNF α (Tomita N., et al., Gene Ther., 2000, Aug; 7(15)1326-32); and the like.

It was suggested that NF-κB suppresses MMP1 and MMP9, members of matrix metalloproteinase (MMP), at the transcription level (Eberhardt W., Huwiler A., Beck K. F., Walpen S., Pfeilschifter J., "Amplification of IL-1β-induced matrix metalloproteinase-9 expression by superoxide in rat glomerular mesangial cells is mediated by increased activities of NF-κB and activating protein-1 and involves activation of the mitogen-activated protein kinase pathways", J. Immunol., 2000, Nov 15, 165(10), 5788-97; Bond M., Baker A. H., Newby A. C., "Nuclear factor κB activity is essential for matrix metalloproteinase-1 and -3 upregulation in rabbit dermal fibroblasts", Biochem. Biophys. Res. Commun., 1999, Oct 22, 264(2), 561-7; Bond M., Fabunmi R. P., Baker A. H., Newby A. C., "Synergistic upregulation of metalloproteinase-9 by growth factors and inflammatory cytokines: an absolute requirement for transcriptional factor NF-κB", FEBS Lett., 1998, Sep 11, 435(1), 29-34; and Kim H., Koh G., "Lipopolysaccharide activates matrix metalloproteinase-2 in endothelial cells through an NF-κB-dependent pathway", Biochem. Biophys. Res. Commun., 2000, Mar 16, 269(2), 401-5).

MMP is a polygene family of zinc-dependent enzymes involved in degradation of extracellular matrix components. It is also known that ets suppresses MMP1 and MMP9, members of matrix metalloproteinase (MMP), at the transcription level (Sato Y., Abe M., Tanaka K., Iwasaka C., Oda N., Kanno S., Oikawa M., Nakano T., Igarashi T., "Signal transduction and transcriptional regulation of angiogenesis", Adv. Exp. Med. Biol., 2000, 476, 109-15; and Oda N., Abe M., Sato Y., "ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin β3", J. Cell Physiol., 1999, Feb, 178(2), 121-32).

MMP plays an important role in invasion of cancer cells by mediating degradation of extracellular matrix protein. A number of studies suggested the involvement of MMP and MMP inhibitors (TIMP) in the progression of cancer: the TIMP1 level in serum may be used as a marker for prognosis and diagnosis of colon and rectum cancer, and as a selective marker for metastatic cancer (Pellegrini P., et al., Cancer Immunol. Immunother., 2000 Sep; 49(7):388-94); expression and activity of MMP2 and MMP9 in human urinary bladder cancer cells are affected by tumor necrosis factor α and γ interferon (Shin KY et al., Cancer Lett 2000 Oct 31; 159(2):127-134); MMP2, MMP9 and MT1-MMP, and their inhibitors, TIMP1 and TIMP2, are expressed in ovarian epithelium tumor (Sakata K., et al., Int. J. Oncol., 2000, Oct; 17(4):673-681); the level of each of MMP1, MMP2, MMP3 and MMP9 and the overall MMP activity are upregulated in colon and rectum tumor, and MMP1 is most important for progression of colon and rectum cancer (Baker E. A., et al., Br. J. Surg., 2000, Sep; 87(9):1215-1221); activated MMP2 plays an important role in invasion of urothelial cancer, and also the expression level of the activated MMP2 can be used as a useful prognosis index (Kaneda K., et al., BJU Int., 2000, Sep; 86(4):553-557); a prostaglandin synthesis inhibitor inhibits invasion of human prostate tumor cells, and reduces the release of MMP (Attiga F. A., et al., Cancer Res., 2000, Aug 15; 60(16):4629-37); the MMP activity of a serum euglobulin fraction increases in breast cancer and lung cancer patients, and may be used as a tumor marker for these cancers (Farias E., et al., Int. J. Cancer, 2000, Jul 20; 89(4):389-94); a MMP inhibitor inhibits gelatin-degrading activity in tumor cells (Ikeda M., et al., Clin. Cancer Res., 2000, Aug; 6(8):3290-6); induction of MMP9 due to a membrane protein

LMP1 contributes to metastatic of nasopharyngeal cancer (NPC) (Horikawa T., et al., Cancer, 2000, Aug 15; 89(4):715-23); MMP plays an important role in an early stage of angioplasty, and a MMP inhibitor suppresses invasion and morphogenesis of human microvascular endothelial cells (Jia M. C., et al., Adv. Exp. Med. Biol., 2000; 476:181-94); MMP9 is expressed in invasive and recurrent pituitary adenoma and hypophysis cancer (Turner H. E., et al., J. Clin. Endocrinol. Metab., 2000, Aug; 85(8):2931-5); and the like.

MMP is also known to be involved in development of aortic aneurysm: MMP is involved in formation and rupture of cerebral aneurysm (Gaetani P., et al., Neurol. Res., 1999, Jun; 21(4):385-90); a MMP-9 promotor is a risk factor for cerebral aneurysm (Peters D. G., et al., Stroke, 1999, Dec; 30(12):2612-6); inhibition of MMP inhibits the growth of microaneurysm in an aneurysm model (Treharne G. D., et al., Br. J. Surg., 1999, Aug; 86(8):1053-8); and the like. MMP is secreted from migrating vascular smooth muscle cells, macrophage, and the like, and destroys collagen, elastin, and the like present in blood vessel walls, whereby the tension of the blood vessel is lost and the blood vessel does not resist the blood pressure and its diameter is expanded. In fact, in the blood vessel of an aneurysm, significant destruction of elastin is observed (Halloran B. G., Baxter B. T., "Pathogenesis of aneurysms", Semin. Vasc. Surg., 1995, Jun 8, (2):85-92).

Aortic aneurysmal rupture is substantially fatal. To prevent aortic aneurysmal rupture, it is important to remove risk factors of arteriosclerosis. However, it is difficult to eliminate the risk factors. At present, invasive surgery is the only means for preventing aortic

aneurysmal rupture.

According to data obtained by measuring the aorta diameter of from 35-year-old to 80-year old adult males, the average was 1.5 cm to 2.0 cm (Dolores J Katz, James C. Stanley, Gerald B. Zelenock, "Abdominal Aortic Aneurysms", Seminars in Vascular Surgery, vol. 8, No. 4 (Dec), 1995; pp. 289-298). In general, the aorta having a diameter beyond 1.5 times as great as the average value is judged as an aortic aneurysm. However, according to the above-described data, one in every 400 people had an aneurysm having a diameter of 3 cm or more which is judged as aortic aneurysm. Therefore, although the degree of risk of aorta rupture is not considered here, the prevalence of aortic aneurysm is relatively high in from 35-year-old to 80-year old adult males. The prevalence is believed to be even greater in males aged 65 and above.

It is known that MMPs are involved in chronic articular rheumatism: alleviation of chronic articular rheumatism by drug treatment leads to a decrease in MMP1 within synovial membrane tissue (Kraan M. C., et al., Arthritis Rheum., 2000, Aug; 43(8):1820-30); upregulation of MT-MMP expression by IL-1β partially induces activation of MMP-2, leading to cytokine-mediated articular disruption in chronic articular rheumatism (Origuchi T., Clin. Exp. Rheumatol., 2000, May-Jun; 18(3):333-9); inflammatory cytokine IL-17 produced in synovial membrane of chronic articular rheumatism increases production of MMP1 (Chabaud M., et al., Cytokine, 2000, Jul; 12(7):1092-9); MMP1, MMP2, MMP3, MMP8, MMP9 and an MMP inhibitor are present in the chronic articular rheumatism synovia at a high level, when MMPs are activated, the balance with the MMP inhibitor is

lost, resulting in cartilage disruption (Yoshihara Y., et al., Ann. Rheum. Dis., 2000, Jun; 59(6):455-61); MT1-MMP is involved in activation of proMMP-2 in rheumatic synovial membrane lining cell layers, leading to cartilage disruption in chronic articular rheumatism (Yamanaka H., et al., Lab. Invest., 2000, May; 80(5):677-87); and the like.

MMP is involved in cardiovascular lesions due to Marfan's syndrome (Segura A. M., et al., Circulation, 1998, Nov 10; 98(19 Suppl):11331-7).

Expression of membrane type MMP (MT-MMP) is increased in mesangial proliferative glomerulonephritis (Hayashi K., et al., J. Am. Soc. Nephrol., 1998, Dec; 9(12):2262-71).

It has been reported that a MMP inhibitor suppresses the expansion of a blood vessel diameter in an aortic aneurysm model in rat abdomen (Moore G., Liao S., Curci J. A., Starcher B. C., Martin R. L., Hendricks R. T., Chen J. J., Thompson R. W., "Suppression of experimental abdominal aortic aneurysms by systemic treatment with a hydroxamate-based matrix metalloproteinase inhibitor" (RS 132908), J. Vasc. Surg., 1999, Mar; 29(3):522-32).

A MMP inhibitor may be used in therapy for glomerulonephritis (Marti HP, Schweiz Med Wochenschr 2000 May 27; 130(21); 784-8). However, systemic administration of a MMP inhibitor causes severe side effects, and has difficulty in clinical applications for treatment (therapy and prevention) of various diseases.

Although a number of patients suffer from asthma, such as bronchial asthma, allergic asthma, childhood asthma,

steroid-resistant asthma (SRA), and the like, it is difficult to curatively treat the disease. A curative treatment has long been desired. Current therapies rely on symptomatic treatment. When asthmatic attacks occur, patients are often treated by administering a bronchodilator, such as theophylline or the like. However, such symptomatic treatment is only performed after attacks. Therefore, the symptom of the patient cannot be completely prevented, much less cured. Therefore, a therapy for eliminating the causes of asthma has long been desired.

It has been suggested that transcriptional factors, such as NF-κB, are involved in various diseases via expression of a number of genes under the transcription control thereof. However, no method for effectively treating these diseases, particularly a non-invasive treatment method, has been provided. Particularly, as described above, aortic aneurysm is not a rare disease. As society ages, an increase in arteriosclerotic diseases inevitably leads to an increase in aortic aneurysm diseases. Considering the aging of patients, it is ideal to suppress directly the growth of aortic aneurysm using a pharmaceutical agent, however, to date such a means is not present. There is a desperate demand for development of a low-invasive therapy and prevention method for aortic aneurysm. There are a number of patients suffering from various diseases, such as cancer, Marfan's syndrome, aortic detachment, post-angioplasty restenosis, chronic articular rheumatism, asthma, atopic asthma, nephritis, renal failure, plaque rupture, and the like. Most of these diseases have severe symptoms. Therefore, a non-invasive therapeutic and prophylactic method for aortic aneurysm has been desired.

SUMMARY OF THE INVENTION

The present invention provides a composition suitable for treatment of the above-described various diseases caused by expression of a gene controlled by NF-κB or ets, and a method of using the same.

The present invention provides a composition comprising a NF-κB decoy, an ets decoy, or a chimera (double) decoy of NF-κB and ets as a major ingredient, which is used to treat and/or prevent various diseases caused by expression of a gene controlled NF-κB or ets, and a method for treating the diseases.

The present inventors found that administration of a NF-κB or ets decoy or a chimera (double) decoy of NF-κB and ets is effective for treatment of diseases caused by expression of a gene controlled by NF-κB or ets. Thus, the present invention was completed.

According to an aspect of the present invention, a pharmaceutical composition for treatment and/or prevention of a disease, a disorder and/or a condition caused by expression of a gene controlled by NF-κB or ets is provided. The composition comprises at least one decoy and a pharmaceutically acceptable carrier.

In one embodiment of this invention, the decoy is an NF-κB decoy or a derivative, variant or fragment thereof, and the derivative, variant or fragment thereof has biological activity.

In one embodiment of this invention, the decoy is

an NF-κB decoy.

In one embodiment of this invention, the decoy is an ets decoy.

In one embodiment of this invention, the decoy is a chimera decoy of NF-κB and ets.

In one embodiment of this invention, the disease is cerebral aneurysm, cancer, Marfan's syndrome, aortic detachment, post-angioplasty restenosis, chronic articular rheumatism, asthma, atopic dermatitis, nephritis, renal failure, or plaque rupture.

In one embodiment of this invention, the pharmaceutically acceptable carrier is a hydrophilic polymer.

In one embodiment of this invention, the pharmaceutically acceptable carrier is a liposome.

According to another aspect of the present invention, a pharmaceutical composition for treatment and/or prevention of a disease, a disorder and/or a condition caused by eosinophilic abnormality is provided. The composition comprises at least one decoy and a pharmaceutically acceptable carrier.

In one embodiment of this invention, the decoy is an NF-κB decoy or a derivative, variant or fragment thereof, and the derivative, variant or fragment thereof has biological activity.

In one embodiment of this invention, the decoy is an NF-κB decoy.

In one embodiment of this invention, the decoy is an ets decoy.

In one embodiment of this invention, the decoy is an NF-κB decoy or an ets chimera decoy.

In one embodiment of this invention, the disease is selected from the group consisting of asthma, allergic diseases, skin diseases, and mycosis.

In one embodiment of this invention, the disease is selected from the group consisting of bronchial asthma, childhood asthma, allergic asthma, atopic asthma, steroid-resistant asthma (SRA), non-allergic asthma, intrinsic asthma, extrinsic asthma, aspirin-induced asthma, cardiac asthma, and infectious asthma.

In one embodiment of this invention, the pharmaceutically acceptable carrier is a hydrophilic polymer.

In one embodiment of this invention, the pharmaceutically acceptable carrier is a liposome.

According to another aspect of the present invention, the present invention relates to use of at least one decoy for treatment and/or prevention of diseases, disorders, and/or conditions caused by expression of a gene controlled NF-κB or ets; treatment and/or prevention of diseases, disorders, and/or conditions caused by eosinophilic

abnormality; or preparation of a pharmaceutical composition for the above-described treatment and/or prevention.

These and other advantages of the present invention will become apparent to those skilled in the art upon reading and understanding the following detailed description with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows light micrographs of the most expanded portions of the human aorta.

Figure 2 shows light micrographs of the most expanded portions of the human aorta.

Figure 3 shows light micrographs of the most expanded portions of the human aorta.

Figure 4 shows the result of a test using the pharmaceutical composition of the present invention.

Figure 5 shows the result of a test using the pharmaceutical composition of the present invention.

Figure 6 shows the result of a test using the pharmaceutical composition of the present invention.

Figure 7 shows the result of a test using the pharmaceutical composition of the present invention.

Figure 8 shows fluorescent micrographs of cross sections of the rat abdominal aorta wall indicating the result

of a test using the pharmaceutical composition of the present invention.

Figure **9** shows fluorescent micrographs of cross sections of the rat abdominal aorta wall indicating the result of a test using the pharmaceutical composition of the present invention.

Figure **10** shows the result of a test using the pharmaceutical composition of the present invention.

Figure **11** shows experimental protocols indicating an effect of the pharmaceutical composition of the present invention using an asthma model (Figure **11A**). Figures **11B** and **11C** show how the decoys of the invention were introduced in a subject.

Figure **12** shows the result indicating an effect of the pharmaceutical composition of the present invention in a bronchial alveolar lavage fluid in ovalbumin-challenged animals. Figures **12A** and **12B** show the fundamental data for eosinophil counts. Figure **12C** shows effects of the invention. From the left, the bars indicate control, ovalbumin challenge, aerosol treatment (2 mg × 2), and liquid treatment (0.5 mg × 1).

Figure **13** shows the result indicating an effect of the pharmaceutical composition of the present invention in a bronchial alveolar lavage fluid in ovalbumin-challenged animals. Figures **13A** (eosinophil cell counts) and **13B** (eosinophil %) show effects of the invention. From the left, the bars indicate control, ovalbumin challenge, naked decoy 500 μg, HVJ (200) (containing 10000 HAU of HVJ-E and 200 μg

of decoy), HVJ(500) (containing 25000 HAU of HVJ-E and 500 μg of decoy), and vector (containing only 25000 HAU of HVJ-E, no decoy). # indicates the statistical significance of ovalbumin challenge.

Figure **14** shows an exemplary behavior of eosinophils by decoy treatments.

These and other advantages of the present invention will become apparent to those skilled in the art upon reading and understanding the following detailed description with reference to the accompanying figures.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

It should be understood throughout the present specification that expression of a singular form includes the concept of their plurality unless otherwise mentioned. Specifically, articles for a singular form (e.g., "a", "an", "the", etc.) include the concept of their plurality unless otherwise mentioned. It should be also understood that the terms as used herein have definitions typically used in the art unless otherwise mentioned. Thus, unless otherwise defined, all scientific and technical terms have the same meanings as those generally used by those skilled in the art to which the present invention pertain. If there is contradiction, the present specification (including the definition) takes precedence.

The embodiments provided below are for better understanding of the present invention. The scope of the present invention is not limited by the embodiments. Therefore, it is clearly understood by those skilled in the

art from the present specification that modifications and variations can be made without departing from the scope of the present invention.

The term "decoy" or "decoy compound" refers to a compound which binds to a site on a chromosome, which NF-κB or ets binds to, or a site on a chromosome, which another transcription regulatory factor for a gene controlled by NF-κB or ets (hereinafter referred to as a target binding site) binds to, and antagonizes the binding of NF-κB, ets, or other transcriptional factors to these target binding sites. Representatively, the decoy or the decoy compound includes a nucleic acid and analogs thereof.

When a decoy is present within a nucleus, the decoy conflicts with a transcription regulatory factor competing for a target binding site for the transcription regulatory factor. As a result, a biological function which would be generated by binding of the transcription regulatory factor to the target binding site is inhibited. The decoy contains at least one nucleic acid sequence capable of binding to a target binding sequence. A decoy can be used for preparation of a pharmaceutical composition according to the present invention as long as the decoy can bind to a target binding sequence.

Preferable examples of a decoy include, but are not limited to, 5'-CCT-TGA-AGG-GAT-TTC-CCT-CC-3' (SEQ ID NO. 1) (NF-κB decoy); 5'-AAT-TCA-CCG-GAA-GTA-TTC-GA-3' (SEQ ID NO. 3) (ets decoy); 5'-ACC-GGA-AGT-ATG-AGG-GAT-TTC-CCT-CC-3' (SEQ ID NO. 5) (chimera (double) decoy of NF-κB and ets); an oligonucleotide containing a complement thereof; a variant thereof; and a

compound including one or more of these molecules. Other preferable examples of a decoy include, but are not limited to, 5'-GATCTAGGGATTTCCGGGAAATGAAGCT-3' (SEQ ID NO. 7) (STAT-1 decoy); 5'-AGCTTGAGATAGAGCT-3' (SEQ ID NO. 8) (GATA-3 decoy); 5'-GATCAAGACCTTTTCCCAAGAAATCTAT-3' (SEQ ID NO. 9) (STAT-6 decoy); 5'-AGCTTGTGAGTCAGAAGCT-3' (SEQ ID NO. 10) (AP-1 decoy); 5'-TGACGTCA-3' (CRE decoy sequence) (SEQ ID NO. 11); 5'-CTAGATTTCCCGC-3' (E2F decoy sequence) (SEQ ID NO. 12); an oligonucleotide containing a complement thereof; a variant thereof; and a compound containing one or more of these molecules. The oligonucleotides may also include a modified nucleic acid (derivative nucleotide) and/or pseudonucleic acid (derivative oligonucleotide) therein. Further, these oligonucleotides may be mutants thereof, or compounds containing them therein. The oligonucleotides may have a single strand or double strands, or may be linear or annular, or may be of a branched chain or of a straight chain. The mutants are nucleic acids having the above-described sequences, a part of which has a mutation, a substitution, an insertion, or a deletion, and which specifically antagonize NF-κB, or another transcription regulatory factor for a gene controlled by NF-κB, with respect to the nucleic acid binding site to which the factor binds. More preferable examples of the decoy for NF-κB and ets, or the other transcription regulatory factor for a gene controlled by NF-κB, include double-strand oligonucleotides containing one or a plurality of the above-described nucleic acid sequences, or mutants thereof. Nucleic acids containing one or a plurality of the above-described nucleic acid sequences are called chimera (double) decoy when the number of nucleic acid sequences contained is two, or triple decoy when the number of nucleic acid sequences contained is three, indicating the number of nucleic acid sequences.

The oligonucleotides for use in the present invention include oligonucleotides modified so as to resist in vivo degradation, and the like, such as oligonucleotides (S-oligo) having a thiophosphatediester bond which is a phosphatediester bond whose oxygen atom is replaced with a sulfur atom, oligonucleotides whose phosphatediester bond is substituted with a methylphosphate group having no electronic charge, and the like.

Biological techniques, biochemical techniques, and microbiological techniques used herein are well known and commonly used in the art, and described in, for example, Ausubel F.A. et al. ed (1988), Current Protocols in Molecular Biology, Wiley, New York, NY; Sambrook J et al. (1987) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Jikken-Igaku "Idenshi-Donyu & Hatsugen-Kaiseki-Jikkenho [Experimental medicine "Experimental methods for Gene introduction & Expression Analysis", Yodo-sha, special issue, 1997; and the like.

As used herein, "nucleic acid", "nucleic acid molecule", "polynucleotide", and "oligonucleotide" are herein used interchangeably to refer to a macromolecule (polymer) comprising a series of nucleotides, unless otherwise specified. A nucleotide refers to a nucleoside whose base is a phosphoric ester. The base of the nucleotide is a pyrimidine or purine base (pyrimidine nucleotide and purine nucleotide). Polynucleotides include DNA or RNA.

Further, sequences obtained by homology search through a genetic information database, such as GenBank

(genome data by the human genome project) using software, such as BLAST, based on the sequence of the decoy of the present invention, also fall within the scope of the present invention. Libraries created based on such a database may be subjected to biological screening (e.g., under stringent conditions). Resultant sequences fall within the scope of the present invention.

As used herein, comparison of identity, homology, and similarity of base sequences is performed with a sequence analysis tool, FASTA, using default parameters, unless otherwise mentioned.

As used herein, "polynucleotides hybridizing under stringent conditions" refers to conditions commonly used and well known in the art. Such a polynucleotide can be obtained by conducting colony hybridization, plaque hybridization, Southern blot hybridization, or the like using a polynucleotide selected from the polynucleotides of the present invention. Specifically, a filter on which DNA derived from a colony or plaque is immobilized is used to conduct hybridization at 65°C in the presence of 0.7 to 1.0 M NaCl. Thereafter, a 0.1 to 2-fold concentration SSC (saline-sodium citrate) solution (1-fold concentration SSC solution is composed of 150 mM sodium chloride and 15 mM sodium citrate) is used to wash the filter at 65°C. Polynucleotides identified by this method are referred to as "polynucleotides hybridizing under stringent conditions". Hybridization can be conducted in accordance with a method described in, for example, Molecular Cloning 2nd ed., Current Protocols in Molecular Biology, Supplement 1-38, DNA Cloning 1: Core Techniques, A Practical Approach, Second Edition, Oxford University Press (1995), and the like. Here,

sequences hybridizing under stringent conditions exclude, preferably, sequences containing only A or T.

When particularly specified, the term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.0015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42°C. See Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory, 1989); Anderson et al., Nucleic Acid Hybridization: A Practical Approach Ch. 4 (IRL Press Limited). More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agents) may be optionally used. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate ($NaDodSO_4$ or SDS), Ficoll, Denhardt's solution, sonicated salmon sperm DNA (or another noncomplementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are ordinarily carried out at pH 6.8-7.4; however, at typical ionic strength conditions, the rate of hybridization is

nearly independent of pH. See Anderson et al., Nucleic Acid Hybridization: A Practical Approach Ch. 4 (IRL Press Limited).

Factors affecting the stability of DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by those skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

$$Tm\ (^{\circ}C) = 81.5 + 16.6\ (\log[Na^{+}]) + 0.41\ (\%\ G+C) - 600/N - 0.72\ (\%\ \text{formamide})$$

where N is the length of the duplex formed, $[Na^{+}]$ is the molar concentration of the sodium ion in the hybridization or washing solution, % G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

When particularly specified, the term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015 M sodium chloride, 0.0015 M sodium citrate at 50-65°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 20% formamide at 37-50°C. By way of example, hybridization in "moderately stringent conditions" of 50°C in 0.015 M sodium ion will allow about a 21% mismatch.

It will be appreciated by those skilled in the art that there is no absolute distinction between "highly stringent conditions" and "moderately stringent conditions". For example, at 0.015 M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences, those skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1 M NaCl for oligonucleotide probes up to about 20 nucleotides is given by:

Tm = (2°C per A-T base pair) + (4°C per G-C base pair).

Note that the sodium ion concentration in 6X salt sodium citrate (SSC) is 1 M. See Suggs et al., Developmental Biology Using Purified Genes 683 (Brown and Fox, eds., 1981).

Nucleic acids for use in decoys, such as NF-κB and the like, may be readily isolated from a cDNA library having PCR primers and hybridization probes containing part of a nucleic acid sequence as set forth in, for example, any of SEQ ID NOs. 1, 3, 5, and 7 to 13, or variants thereof. A preferable nucleic acid for use in decoys, such as NF-κB and the like, is hybridizable to the whole or part of a sequence as set forth in any of SEQ ID NOs. 1, 3, 5, and 7 to 13 under low stringency conditions defined by hybridization buffer essentially containing 1% bovine serum albumin (BSA); 500 mM sodium phosphate ($NaPO_4$); 1mM EDTA; and 7% SDS at 42°C, and wash buffer essentially containing 2×SSC (600 mM NaCl; 60 mM sodium citrate); and 0.1% SDS at 50°C, more preferably under

low stringency conditions defined by hybridization buffer essentially containing 1% bovine serum albumin (BSA); 500 mM sodium phosphate ($NaPO_4$); 15% formamide; 1 mM EDTA; and 7% SDS at 50°C, and wash buffer essentially containing 1×SSC (300 mM NaCl; 30 mM sodium citrate); and 1% SDS at 50°C, and most preferably under low stringency conditions defined by hybridization buffer essentially containing 1% bovine serum albumin (BSA); 200 mM sodium phosphate ($NaPO_4$); 15% formamide; 1 mM EDTA; and 7% SDS at 50°C, and wash buffer essentially containing 0.5×SSC (150 mM NaCl; 15 mM sodium citrate); and 0.1% SDS at 65°C.

Therefore, the present invention is intended to encompass sequences which are hybridizable to a specific sequence of the present invention (e.g., any one of SEQ ID NOs. 1, 3, 5, and 7 to 13) under stringent conditions. The stringent conditions may be, for example, highly stringent conditions, moderately stringent conditions, or low stringency conditions. Those skilled in the art can appropriately select these conditions depending on the purpose. In one illustrative embodiment, the present invention is intended to encompass a nucleic acid molecule having a sequence hybridizable to a specific sequence of the present invention (e.g., any one of SEQ ID NOs. 1, 3, 5, and 7 to 13) under stringent conditions.

"Homology" of genes refers to the degree of the identity between two or more gene sequences. Therefore, the greater the homology between certain two genes, the greater the identity or similarity between their sequences. Whether or not two genes have homology, can be studied by comparing two sequences directly or by hybridization under stringent conditions. When two gene sequences are directly compared

to each other, the genes have homology if representatively at least 50%, preferably at least 70%, more preferably at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% of the DNA sequence of the genes are identical.

As used herein, "fragment" of a nucleic acid molecule refers to a polynucleotide having a length which is shorter than the full length of the reference nucleic acid molecule but sufficient for use at least as a factor in the present invention. Therefore, the fragment as used herein refers to a polynucleotide having a sequence length ranging from 1 to n-1 with respect to the full length of the reference polynucleotide (the length is n). The length of the fragment can be appropriately changed depending on the purpose. For example, in the case of polynucleotides, the lower limit of the length of the fragment includes 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100 and more nucleotides. Lengths represented by integers which are not herein specified (e.g., 11 and the like) may be appropriate as a lower limit.

"Hybridizable polynucleotide" refers to a polynucleotide which can hybridize other polynucleotides under the above-described hybridization conditions. Specifically, the hybridizable polynucleotide includes at least a polynucleotide having a homology of at least 60% to the base sequence of SEQ ID NOs: 1, 3, 5, and 7 to 13, preferably a polynucleotide having a homology of at least 80%, and more preferably a polynucleotide having a homology of at least 95%. Homology as described herein is represented by a score using the search program BLAST which employs an algorithm developed by Altschul et al. (J. Mol. Biol. 215, 403-410(1990)), for example. Therefore, unless otherwise mentioned, the similarity, identity and homology of amino

acid sequences and base sequences are herein compared using FASTA (sequence analyzing tool) with the default parameters.

As used herein, "similarity" of a gene (e.g., a nucleic acid sequence, an amino acid sequence, or the like) refers to the proportion of identity between two or more sequences when conservative substitution is regarded as positive (identical) in the above-described homology. Therefore, homology and similarity differ from each other in the presence of conservative substitutions. If no conservative substitutions are present, homology and similarity have the same value.

As used herein, the "percentage of (amino acid, nucleotide, or the like) sequence identity, homology or similarity" is determined by comparing two optimally aligned sequences over a window of comparison, wherein the portion of a polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e. gaps), as compared to the reference sequences (which does not comprise additions or deletions (if the other sequence includes an addition, a gap may occur)) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity. When used in a search, homology is evaluated by an appropriate technique selected from various sequence comparison algorithms and programs well known in the art. Examples of such algorithms and programs include, but are not limited

to, TBLASTN, BLASTP, FASTA, TFASTA and CLUSTALW (Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85(8):2444-2448, Altschul et al., 1990, J. Mol. Biol. 215(3):403-410, Thompson et al., 1994, Nucleic Acids Res. 22(2):4673-4680, Higgins et al., 1996, Methods Enzymol. 266:383-402, Altschul et al., 1990, J. Mol. Biol. 215(3):403-410, Altschul et al., 1993, Nature Genetics 3:266-272). Therefore, in a particular embodiment, the present invention encompasses sequences having a value greater than or equal to a particular value of identity or similarity described herein, which are retrieved using the above-described sequence analysis tools.

As used herein, the term "corresponding" gene or sequence (e.g., a decoy, a promoter sequence, or the like) refers to a gene in a given species, which has, or is anticipated to have, a function similar to that of a predetermined gene or sequence in a species as a reference for comparison. When there are a plurality of genes or sequences having such a function, the term refers to a gene or sequence having the same evolutionary origin. Therefore, a gene or sequence corresponding to a given gene or sequence may be an ortholog of the given gene or sequence. Therefore, genes or sequences corresponding to mouse NF-κB, ets, STAT-1 genes or sequences and the like can be found in other animals (human, rat, pig, cattle, and the like). Such a corresponding gene can be identified by a technique well known in the art. Therefore, for example, a corresponding gene or sequence in a given animal can be found by searching a sequence database of the animal (e.g., human, rat) using the sequence of a reference gene (e.g., a mouse NF-κB sequence or the like) as a query sequence. Thus, the present invention is intended to encompass sequences corresponding to a specific sequence of the present invention (e.g., any one of SEQ ID NOs. 1,

3, 5, and 7 to 13).

"Oligonucleotide derivative" or "polynucleotide derivative" refers to an oligonucleotide including a derivative of a nucleotide or having a linkage between nucleotides which is not normal. Specifically, examples of such an oligonucleotide include an oligonucleotide derivative in which a phosphodiester bond is converted to a phosphothioate bond, an oligonucleotide derivative in which phosphodiester bond is converted to N3'-P5' phosphoramidate bond, an oligonucleotide derivative in which ribose and phosphodiester bond are converted to peptide-nucleic acid bond, an oligonucleotide derivative in which uracil is substituted with C-5 propynyl uracil, an oligonucleotide derivative in which uracil is substituted with C-5 thiazole uracil, an oligonucleotide derivative in which cytosine is substituted with C-5 propynyl cytosine, an oligonucleotide derivative in which cytosine is substituted with phenoxazine-modified cytosine, an oligonucleotide derivative in which ribose is substituted with 2'-O-propynyl ribose, an oligonucleotide derivative in which ribose is substituted with 2'-methoxyethoxy ribose, and the like.

As used herein, "biological activity" refers to the activity which a certain factor (e.g., polynucleotide or polypeptide) has within an organism, including activity exhibiting various functions. For example, when the certain factor is a transcriptional factor, its biological activity includes activity to regulate transcriptional activity. When the certain factor is an enzyme, its biological activity includes enzymatic activity. As another example, when the certain factor is a ligand, its biological activity includes binding to a receptor to which the ligand corresponds. In

one embodiment of the present invention, its biological activity includes activity to bind to at least one transcriptional factor. A method for measuring binding activity of such a transcriptional factor may be achieved by mixing sequences capable of binding a transcriptional factor and the transcriptional factor and measuring resultant complexes (e.g., observation using electrophoresis). Such a method is well known to, and is commonly used by, those skilled in the art.

As used herein, "nucleotide" refers to any naturally occurring nucleotide and non-naturally occurring nucleotide. "Derived nucleotide" refers to a nucleotide which is different from naturally occurring nucleotides but has a function similar to that of its original naturally occurring nucleotide. Such derived nucleotides are well known in the art. Examples of the nucleotide derivative and nucleotide analog include, but are not limited to, phosphorothioate, phosphoramidate, methylphosphonate, chiral methylphosphonate, 2-O-methylribonucleotide, and peptide nucleic acid (PNA).

As used herein, "variant" refers to a substance, such as polynucleotide, or the like, which differs partially from the original substance. Examples of such a variant include a substitution variant, an addition variant, a deletion variant, a truncated variant, an allelic variant, and the like. Allele refers to a genetic variant located at a locus identical to a corresponding gene, where the two genes are distinguished from each other. Therefore, "allelic variant" refers to a variant which has an allele relationship with a certain gene. "Homolog" of a nucleic acid molecule refers to a nucleic acid molecule having a nucleotide sequence

having homology with the nucleotide sequence of a reference nucleic acid molecule. Representatively, "homolog" refers to a polynucleotide which hybridizes to a reference nucleic acid molecule under stringent conditions. In the case of the nucleic acid molecule of the present invention, a "homolog" is a nucleic acid molecule having a nucleic acid sequence having homology with the nucleic acid sequence of the decoy of the present invention, whose biological function is the same as or similar to the promoter of the present invention. Therefore, the concepts of "homolog" and "variant" overlap partially. Therefore, a homolog has amino acid or nucleotide homology with a certain gene in a certain species (preferably at least 60% homology, more preferably at least 80%, at least 85%, at least 90%, and at least 95% homology). A method for obtaining such a homolog is clearly understood from the description of the present specification. For example, a homolog of the decoy of the present invention may be a homologous gene in the same species or a corresponding gene in other species. Therefore, the decoy of the present invention may include all homologs of the decoy.

As used herein, the term "isolated" biological agent (e.g., nucleic acid, protein, or the like) refers to a biological agent that is substantially separated or purified from other biological agents in cells of a naturally-occurring organism (e.g., in the case of nucleic acids, agents other than nucleic acids and a nucleic acid having nucleic acid sequences other than an intended nucleic acid; and in the case of proteins, agents other than proteins and proteins having an amino acid sequence other than an intended protein). The "isolated" nucleic acid and protein include nucleic acids and proteins purified by a standard purification method. The isolated nucleic acids and

proteins also include chemically synthesized nucleic acids and proteins. Therefore, in one embodiment, the nucleic acid of the present invention may be an isolated nucleic acid or the like.

The decoy of the present invention can be produced with chemical or biochemical synthesis methods known in the art. For example, when a nucleic acid is used as a decoy compound, nucleic acid synthesis methods commonly used in genetic engineering can be employed. For example, a DNA synthesizer may be used to directly synthesize intended decoy nucleic acids. Further, these nucleic acids, nucleic acids containing the nucleic acids, or parts thereof may be synthesized, followed by amplification using a PCR method, a cloning vector, and the like. Furthermore, nucleic acids obtained by these methods are cleaved using a restriction enzyme, or the like, and linked or the like using DNA ligase, or the like to produce an intended nucleic acid. To obtain decoy nucleic acids which are more stable in cells, base, sugar and phosphate portions of the nucleic acids may be subjected to chemical modification, such as alkylation, acylation, or the like.

The present invention provides a pharmaceutical composition comprising the above-described decoy compound alone or in combination with a stabilizing compound, a diluent, a carrier or another component, or a pharmaceutical agent.

As used herein, the term "subject" or "patient" refers to an organism to which the treatment or composition of the present invention is applied. The subject or patient may be any animal (e.g., primates, rodents, etc.) as long as the treatment or composition of the present invention is

applicable to them. The subject or patient may be preferably a human.

As used herein, "disease(s), disorder(s), and/or condition(s) caused by expression of a gene controlled by NF-κB or ets" refers to any disease, disorder, or condition which is caused by regulation (e.g., increase, decrease, delay, etc.) of expression (e.g., translation, transcription, etc.) of a transcriptional factor, NF-κB or ets. Examples of such a disease, disorder or condition include, but are not limited to, cerebral aneurysm, cancer, Marfan's syndrome, aortic detachment, post-angioplasty restenosis, chronic articular rheumatism, asthma, atopic dermatitis, nephritis, renal failure, or plaque rupture.

As used herein, "disease(s), disorder(s), and/or condition(s) caused by eosinophilic abnormality" refers to any disease, disorder, or condition, in which an abnormal level of eosinophils in the body (e.g., blood) is a cause or an effect. It is known that eosinophils have an IgE receptor on the surface thereof, particularly functioning in allergic diseases. Eosinophils also have anti-inflammatory action, functioning as immune regulatory cells..

Examples of diseases involved in eosinophilic abnormality include, but are not limited to, for example, asthma (bronchial asthma, childhood asthma, allergic asthma, atopic asthma, steroid-resistant asthma (SRA), non-allergic asthma (intrinsic asthma, extrinsic asthma, (e.g., aspirin-induced asthma, cardiac asthma, infectious asthma)), other eosinophilic abnormality (e.g., mycosis, hemodyscrasia (e.g., chronic myelocytic leukemia, Hodgkin's

disease)), allergic diseases (e.g., urticaria, drug allergy, angiitis, dermatomyositis, hay fever; bronchial asthma can be said to be an allergic disease), parasite diseases (e.g., ascarid, Ancylostoma duodenale, distome, filaria), skin diseases (e.g., pemphigus, prurigo, erythema exsudativum multiforme), malignant diseases (e.g., arteritis nodosa, eosinophilic granuloma, PIE syndrome, Loeffler's syndrome, eosinophilic leukemia), ulcerative colitis, regional enteritis, sarcoidosis, tuberculosis, Addison's disease, Hypereosinophilic syndrome, abnormalities due to drug administration (e.g., a gold agent, capreomycin, amphotericin B).

To use the pharmaceutical composition and method of the present invention for a human subject, the composition is tested, *in vitro* and then *in vivo*, in an appropriate amount at the animal level before use in a human for the purpose of desired treatment or prevention. Effects of the composition to cell lines and/or animal models may be determined in preliminary tests using techniques known to those skilled in the art. An *in vitro* assay for determining the delivery of a specific administered compound according to the present invention, may be performed by observing the binding of a transcriptional factor to a transcriptional factor binding sequence, for example. At the animal level test, a composition is administered as in the case of a human and the level of a therapeutic effect is confirmed (e.g., recovery from aneurysm, fluctuations in eosinophil, etc.). It is usually understood in the art that if a therapeutic and/or prophylactic effect is obtained in a model animal, a similar effect is obtained in a human.

The pharmaceutical composition of the present

invention may be used in such a form that the decoy is taken into cells in an affected part or cells in an intended tissue.

The pharmaceutical composition of the present invention is administered in any aseptic biocompatible pharmaceutical carrier (including, but not limited to, physiological saline, buffered physiological saline, dextrose, and water). A pharmaceutical composition of any of these molecules mixed with an appropriate excipient, an adjuvant, and/or a pharmaceutically acceptable carrier may be administered to patients alone or in combination with another pharmaceutical agent in a pharmaceutical composition. In an embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inactive.

The administration of the pharmaceutical composition of the present invention is achieved orally or parenterally. Parenteral delivery methods include topical, intra-arterial (e.g., directly into tumor, aneurysm, etc.), intramuscular, subcutaneous, intramedullary, into subarachnoid space, intraventricular, intravenous, intraperitoneal, or intranasal administrations. In addition to a decoy compound, the pharmaceutical composition comprises a pharmaceutically acceptable carrier, such as an excipient and other compounds for accelerating the processing of the decoy compound so as to prepare a pharmaceutically acceptable formulation. The further details of techniques for prescription and administration are described in, for example, the latest version of "REMINGTON'S PHARMACEUTICAL SCIENCES" (Maack Publishing Co., Easton, PA).

A pharmaceutical composition for oral administration may be prepared using a pharmaceutically acceptable carrier well known in the art in an administration form suitable for administration. Such a carrier can be prepared as a tablet, a pill, a sugar-coated agent, a capsule, a liquid, a gel, a syrup, a slurry, a suspension, or the like, which is suited for the patient to take the pharmaceutical composition.

The pharmaceutical composition for oral use may be obtained in the following manner: an active compound is combined with a solid excipient, the resultant mixture is pulverized if necessary, an appropriate compound is further added if necessary to obtain a tablet or the core of a sugar-coated agent, and the granular mixture is processed. The appropriate excipient may be a carbohydrate or protein filler, including, but not being limited to, the following: sugar including lactose, sucrose, mannitol, or sorbitol; starch derived from maize, wheat, rice, potato, or other plants; cellulose such as methylcellulose, hydroxypropylmethylcellulose, or sodium carboxymethylcellulose; and gum including gum Arabic and gum tragacanth; and protein such as gelatin and collagen. A disintegrant or a solubilizing agent such as crosslinked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof (e.g., sodium alginate) may be used if necessary.

The sugar-coated agent core is provided along with an appropriate coating, such as a condensed sugar solution. The sugar-coated agent core may also contain gum arabic, talc, polyvinyl pyrrolidone, carbopolygel, polyethylene glycol, and/or titanium dioxide, a lacquer solution, and an appropriate organic solvent or a solvent mixed solution.

To identify a product, or characterize the amount of an active compound (i.e., dose), dye or pigment may be added to tablets or sugar-coated agents.

The pharmaceutical preparation which may be orally used may contain, for example, a soft sealed capsule consisting of a gelatin capsule, gelatin and coating (e.g., glycerol or sorbitol). The gelatin capsule may contain an active component mixed with a filler or binder such as lactose or starch, a lubricant such as talc or magnesium stearate, and optionally a stabilizer. In the soft capsule, the decoy compound may be dissolved or suspended in an appropriate liquid, such as fatty oil, liquid paraffin or liquid polyethylene glycol, with or without a stabilizer.

The pharmaceutical preparation for parenteral administration contains an aqueous solution of an active compound. For the purpose of injection, the pharmaceutical composition of the present invention is prepared in an aqueous solution, preferably Hank's solution, Ringer's solution, or a physiologically suitable buffer such as a buffered physiological saline. The aqueous suspension for injection may contain a substance for increasing the viscosity of a suspension (e.g., sodium carboxymethylcellulose, sorbitol, or dextran). Further, the suspension of the active compound may be prepared as an appropriate oily suspension. Appropriate lipophilic solvents or vehicles include fatty acid such as sesame oil, synthetic fatty acid ester such as ethyl oleate or triglyceride, or liposome. The suspension may contain a stabilizer which allows a high-concentration solution preparation, or an appropriate pharmaceutical agent or reagent for increasing the solubility of the compound, if necessary.

For topical or intranasal administration, an appropriate penetrant for the specific barrier to be penetrated may be used in the preparation. Such a penetrant is generally known in the art.

The pharmaceutical composition of the present invention may be produced using a method similar to method known in the art (e.g., conventional mixing, dissolution, rendering to granules, preparation of a sugar-coated agent, elutriation, emulsification, capsulation, inclusion, or freeze drying).

Preferably, in the case of parenteral administration, such as topical administration or infusion from a cervical portion to cell of an affected part or cells of an intended tissue, the pharmaceutical composition of the present invention may contain a synthetic or naturally-occurring hydrophilic polymer as a carrier. Examples of such a hydrophilic polymer include hydroxypropylcellulose and polyethylene glycol. The decoy compound of the present invention may be mixed with the above-described hydrophilic polymer in an appropriate solvent. The solvent may be removed by a method such as air drying. The resultant compound may be shaped into a desired form, such as sheet, and then may be given to a target site. Such a preparation containing a hydrophilic polymer has a small moisture content, and an excellent shelf life, and an excellent retentivity of the decoy compound since the preparation absorbs water to be turned into gel when used.

Such a sheet may include a hydrophilic sheet obtained by mixing polyhydric alcohol with a compound similar to the

above-described composition components, such as cellulose or starch, or a derivative thereof, a synthetic polymer compound or the like and adjusting the hardness of the sheet.

Such a sheet may be placed in a target site under a laparoscope. Currently, laparoscopic surgery has been dramatically developed as a non-invasive technique. By combining the pharmaceutical composition of the present invention with the laparoscope technique, a method for treatment of diseases, which can be repeatedly used, may be provided.

Alternatively, when a nucleic acid or a modification thereof is employed as a decoy, the pharmaceutical composition of the present invention is advantageously used in a form which is generally used in gene introduction methods, such as a membrane fusion liposome preparation using Sendai virus (HVJ) or the like, a liposome preparation using endocytosis or the like, a preparation containing a cationic lipid such as Lipofectamine (Lifetech Oriental) or the like, or a viral preparation using a retrovirus vector, an adenovirus vector, or the like. Particularly, a membrane fusion liposome preparation is preferable.

The liposome preparation is any of the liposome constructs which are a large unilamellar vesicle (LUV), a multilammelar vesicle (MLV), and a small unilamellar vesicle (SUV). The LUV has a particle system ranging from about 200 to about 1000 nm. The MLV has a particle system ranging from about 40 to about 350 nm. The SUV has a particle system ranging from about 20 to about 50 nm. The membrane fusion liposome preparation using Sendai virus or the like preferably employs MLV having a particle system ranging from

200 nm to 1000 nm.

There is no particular limitation on a method for producing liposomes as long as the liposomes hold a decoy. The liposomes can be produced by a commonly used method, such as, for example, a reversed phase evaporation method (Szoka, F et al., Biochim. Biophys. Acta, Vol. 601 559(1980)), an ether infusion method (Deamer, D.W.: Ann. N.Y. Acad. Sci., Vol. 308 250(1978)), a surfactant method (Brunner, J et al.: Biochim. Biophys. Acta, Vol. 455 322(1976)), or the like.

Examples of lipids for forming a structure of a liposome include phospholipids, cholesterols, nitrogen lipids, and the like. Generally, phospholipids are preferable, including naturally-occurring phospholipids, such as phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, cardiolipin, sphingomyelin, egg yolk lecithin, soybean lecithin, lysolecithin, and the like, or the corresponding phospholipids hydrogenated by a commonly used method, and in addition, synthetic phospholipids, such as dicetylphosphate, distearoylphosphatidylcholine, dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylethanolamine, dipalmitoylphosphatidylserine, eleostearoylphosphatidylcholine, eleostearoylphosphatidylethanolamine, eleostearoylphosphatidylserine, and the like.

The lipids including these phospholipids can be used alone or with at least two in a combination. In this case, lipids having an atom group having a positive group, such

as ethanolamine, choline, or the like, within the molecule can be used to increase the binding rate of an electrically negative decoy nucleic acid. In addition to the major phospholipids used to form liposomes, an additive, such as cholesterols, stearylamine, α-tocopherol, or the like, which are generally known as an additive for formation of liposomes, can be used.

The thus-obtained liposomes can additionally contain a substance for promoting membrane fusion, such as a membrane fusion promoting protein purified from Sendai virus, inactivated Sendai virus, Sendai virus, or the like, so as to accelerate uptake into cells at an affected site or cells in an intended tissue.

An exemplary method for producing a liposome preparation will be specifically described below. For example, the above-described substance for forming a liposome is dissolved along with cholesterol in an organic solvent, such as tetrahydrofuran, chloroform, ethanol, or the like. The resultant solution is put into an appropriate vessel, followed by removal of the solvent under reduced pressure, thereby forming a film of the liposome forming substance on an inside wall of the vessel. A buffer solution containing a decoy is added to the vessel followed by agitation. The above-described membrane fusion promoting substance is added to the resultant liposome if necessary, followed by isolation of the liposome. The thus-obtained liposome containing the decoy can be suspended in an appropriate solvent or can be freeze-dried and thereafter dispersed in an appropriate solvent. The resultant suspension can be used in treatment. The membrane fusion promoting substance may be added in the interim period after the isolation of the liposome and before

use.

The pharmaceutical composition of the present invention includes a composition containing an effective amount of decoy compound which can achieve the intended purpose of the decoy compound. "Therapeutically effective amount" or "pharmacologically effective amount" are terms which are well recognized by those skilled in the art and which refer to an amount of pharmaceutical agent effective for production of an intended pharmacological effect. Therefore, the therapeutically effective amount is an amount sufficient for reducing the manifestation of the disease to be treated. A useful assay for confirming an effective amount (e.g., a therapeutically effective amount) for a predetermined application is to measure the degree of recovery from a target disease. An amount actually administered depends on an individual to be treated. The amount is preferably optimized so as to achieve a desired effect without a significant side effect. The determination of the therapeutically effective dose is within the ability of those skilled in the art.

A therapeutically effective dose of any compound can be initially estimated using either a cell culture assay or any appropriate animal model. The animal model is used to achieve a desired concentration range and an administration route. Thereafter, such information can be used to determine a dose and route useful for administration into humans.

The therapeutically effective amount refers to an amount of a decoy compound which results in amelioration of symptoms or conditions of a disease. The therapeutic

effect and toxicity of such a compound may be determined by standard pharmaceutical procedures in cell cultures or experimental animals (e.g., ED_{50}, a dose therapeutically effective for 50% of a population; and LD_{50}, a dose lethal to 50% of a population). The dose ratio between therapeutic and toxic effects is a therapeutic index, and it can be expressed as the ratio of ED_{50}/LD_{50}. Pharmaceutical compositions which exhibit high therapeutic indices are preferable. The data obtained from cell culture assays and animal studies can be used in formulating a dosage range for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. Such a dosage may vary within this range depending upon the dosage form employed, the susceptibility of a patient, and the route of administration. As an example, the dose of a decoy is appropriately selected depending on the age and other conditions of a patient, the type of a disease, the type of the decoy employed, and the like. For example, in the case of intravascular administration, intramuscular administration, and intra-articular administration, 1 μg to 100 mg can be generally administered once a day to several times a day.

The exact dose is chosen by an individual physician in view of the condition of a patient to be treated. Doses and administration are adjusted to provide a sufficient level of the active portion, or to hold a desired effect. Additional factors to be considered include the severity of the condition of a disease (e.g., the size and location of a tumor; the age, weight and sex of a patient; a diet-limiting time and frequency of administration, a combination of drugs, reaction susceptibility, and

resistance/response to treatment). A sustained action pharmaceutical composition may be administered every 3 to 4 days, every week, or once per two weeks, depending on the half life and clearance rate of a specific preparation. Guidance for specific doses and delivery methods are provided in publications known in the art.

Medicaments containing the thus-obtained decoy as a major component can be administered in various manners, depending on the type of disease, the type of the decoy employed, and the like. For example, the medicament can be intravascularly administered, applied to the site of a disease, administered to the disease site, or intravascularly administered to the disease site, for ischemic diseases, inflammatory diseases, autoimmune diseases, and cancer metastasis and invasion, and cachexia. More specifically, for example, when PTCA is performed for infarct of an organ, the medicament can be administered into a blood vessel of an affected part at the same time or before or after the PTCA. In organ transplantation or the like, an organ to be transplanted may be treated in advance with a preparation for use in the present invention. Further, for example, the medicament can be infused directly to a joint in the case of chronic articular rheumatism or the like. Alternatively, for asthma or the like, the composition may be administered by inhalation or insufflation using an inhaler (MDI · BDI), a nebulizer, or the like.

Examples

Hereinafter, the present invention will be described by way of examples. These examples are for illustrative purposes only. The present invention is not limited by these examples, except as by the appended claims.

(Example 1: Overexpression of ets-1 in human aortic aneurysm sample)

Ets-1 is one of the transcriptional factors which regulate expression of the MMP gene. Aortic aneurysm samples were surgically removed (excised) and fixed in formalin. The samples were subjected to commonly used immunostaining using an anti-ets-1 antibody (available from Santa Cruz Biotechnology (USA)). As shown in Figures **1**, **2**, and **3**, the presence of ets-1 was confirmed in all of the aortic aneurysm samples, mainly the adventitia thereof.

A photograph to the left of Figure **1** is a light micrograph showing the human aortic root (× 100). A photograph to the right of Figure **1** is an enlarged photograph (× 400) of a rectangular section in the left photograph.

A photograph to the left of Figure **2** is a light micrograph showing the human aortic root (× 100).

A photograph to the left of Figure **2** is a light micrograph (× 100) of the most expanded portion of the human aorta. A photograph to the right of Figure **2** is a fluorescent micrograph (× 200) of the most expanded portion of the human aorta.

Figure **3** are light micrographs (× 400) of the most expanded portion of the human aorta, which are enlarged photographs of rectangular sections in Figure **2**. The photograph to the left of Figure **3** is an enlarged photograph (× 400) of the larger rectangular section in the right photograph of Figure **3**. The photograph to the left of Figure **3** is an enlarged photograph (× 400) of the smaller

rectangular section in the right photograph of Figure 3.

(Example 2: Effect of Decoy Nucleic Acid in Organ Culture (Tissue Culture))

Aortic aneurysm samples surgically removed were used in organ culture (tissue culture) to test an effect of decoy nucleic acid transfer on suppression of MMP gene expression.

Human aortic aneurysm was surgically removed and divided into 2 mm^2 samples. The samples were immersed in 10% collagen gel containing 100 μM of a decoy or a scrambled decoy (synthesized by Hokkaido System Science) at room temperature for 1 hour. Thereafter, the samples were placed in 24-well plates with the gel being attached to the samples. 1.5 ml of culture medium (Dulbecco's modified Eagle's medium, 1% FCS) was added to each well, followed by culturing at 37°C in an incubator. After 24 hours, the culture medium was removed and new culture medium was added to the plate. After another 48 hours, MMP1 and MMP9 in the culture medium were measured by a commonly used method using ELISA (manufactured by Amersham Pharmacia Biotech).

Decoys used herein:

NF-κB decoy (SEQ ID NO. 1)

5'-CCT-TGA-AGG-GAT-TTC-CCT-CC-3'

3'-GGA-GGG-AAA-TCC-CTT-CAA-GG-5'

NF-κB scrambled decoy (SEQ ID NO. 2)

5'-TTG-CCG-TAC-CTG-ACT-TAG-CC-3'

3'-GGC-TAA-GTC-AGG-TAC-GGC-AA-5'

ets decoy (SEQ ID NO. 3)

5'-AAT-TCA-CCG-GAA-GTA-TTC-GA-3'

3'-TTA-AGT-GGC-CTT-CAT-AAG-CT-5'

ets scrambled decoy (SEQ ID NO. 4)
5'-GGA-ATA-CAT-CGA-CCT-GTT-AA-3'
3'-CCT-TAT-GTA-GCT-GGA-CAA-TT-3'

The results are shown in Figures **4** and **5**. In Figures **4** and **5**, the vertical axis represents absorbance at 450 nm, while "untreat", "NFsd", "NF", "ets-sd", and "ets" on the horizontal axis represent no nucleic acid reagent (control), NF-κB scrambled decoy, NF-κB decoy, ets scrambled decoy, and ets decoy, respectively. In Figures **4** and **5**, a horizontal bar on each bar represents a standard deviation, and "P" on a line connected between each bar represents the level of significance which is used to compare groups connected via the line. "**" on a horizontal bar indicates that the average value of the corresponding group is different from the average of the control at a significance level of 1% (Figure **4**) or 5% (Figure **5**) (Fisher test).

As can be seen from Figures **4** and **5**, in the ets decoy-administered group, production of MMP1 and MMP9 was significantly suppressed as compared to the control group and the ets scrambled decoy-administered group. Also, in the NF-κB decoy-administered group, production of MMP1 and MMP9 was significantly suppressed as compared to the control group and the NF-κB scrambled decoy-administered group.

(Example 3: Concentration-dependent Effect of Decoy Nucleic Acid and Double Decoy Nucleic Acid on Organ Culture (Tissue Culture System))

An effect of decoy nucleic acid addition on suppression of MMP gene expression was tested in organ culture

(tissue culture system) by the same method as in Example 2, except that the added decoy nucleic acids were 100 μM and 600 μM NF-κB decoy, and 100 μM and 600 μM double decoy and double scrambled decoy having the following structure.

Double decoy (SEQ ID NO. 5)
5'-ACC-GGA-AGT-AGA-AGG-GAT-TTC-CCT-CC-3'
3'-TGG-CCT-TCA-TCT-TCC-CTA-AAG-GGA-GG-5'

Double scrambled decoy (SEQ ID NO. 6)
5'-GCA-ACC-CCT-TAG-GTT-CTG-AGA-GAC-GA-3'
3'-CGT-TGG-GGA-ATC-CAA-GAC-TCT-CTG-CT-5'

The results are shown in Figures **6** and **7**. In Figures **6** and **7**, the vertical axis represents absorbance at 450 nm, while "untreat", "NFsd", "NF100", "NF600", "DD sd", "DD100", and "DD600" on the horizontal axis represent no nucleic acid reagent (control), 100 μM NF-κB decoy, 600 μM NF-κB decoy, double scrambled decoy, 100 μM double decoy, and 600 μM double decoy. In Figures **6** and **7**, a horizontal bar on each bar represents a standard deviation, and "P" on a line connected between each bar represents the level of significance which is used to compare groups connected via the line. "*" and "**" indicate that there is a statistically significant difference in average value between the group and the control with a significance level of 5% and 1%, respectively. "#" and "‡" indicate that there is a significant difference in average value between the NF100 or NF600 group and the control with a significance level of 5% (Fisher test).

As can be seen from Figures **6** and **7**, production of MMP1 and MMP9 in the NF-κB-administered group was

significantly suppressed as compared to the control group and the NF-κB scrambled decoy-administered group, and this effect was concentration-dependent. Also, production of MMP1 and MMP9 in the double decoy-administered group was suppressed as compared to the scrambled decoy-administered group. The effect of the double decoy was more significant as compared to the NF-κB decoy-administered group.

(Example 4: In vivo Effect of Decoy Nucleic Acid)

Rats were used to test an effect of decoy nucleic acid *in vivo* administration on suppression of MMP gene expression.

Rats (SD rats, 12 weeks old) were anesthetized and abdominal incisions were performed. The abdominal aorta was wrapped over a length of about 1 cm with AD film (dimensions: 1 cm × 1 cm) described below. The abdominal incisions were closed and the animals were kept in normal situations. After 3 day, the abdominal incisions were performed again to remove blood vessels, followed by fluorescent microscopic analysis.

The composition of the AD film is: hydroxypropyl cellulose 150 to 400 cps (HPC-M) 73 mg/4cm^2; polyethylene glycol 400 (PEG) 7.3 mg/4cm^2; FITC-labeled decoy 100 nmol/cm^2.

Method for preparing the AD film is: initially, the above-described hydroxypropyl cellulose and polyethylene glycol were dissolved in 100% ethanol and mixed together. 400 nmol of the FITC-labeled decoy was added and dissolved in the mixture, followed by air drying, to finally form a sheet of 4 cm^2.

The results are shown in Figures **8** and **9**. Figure **8** shows fluorescent micrographs (× 200) showing partial cross sections of the abdominal aorta walls. A photograph to the left of Figure **8** is a fluorescent micrograph showing a cross section of the abdominal aorta wall of the control rat, which was wrapped with the AD film not containing the FITC-labeled decoy. A photograph to the right of Figure **8** is a fluorescent micrograph showing a cross section of the abdominal aorta wall of the control rat, which was wrapped with the AD film containing the FITC-labeled decoy. Figure **9** shows fluorescent micrographs showing a cross section and a partial cross section of the abdominal aorta wall. A photograph to the left of Figure **9** is a 100-fold magnification fluorescent micrograph. A photograph to the right of Figure **9** is a 200-fold magnification fluorescent micrograph.

As can be seen from Figures **8** and **9**, strong green color fluorescence is observed in the vascular adventitia of the abdominal aorta wall of the rat wrapped with the AD film containing the FITC-labeled decoy, and green fluorescence is observed in the media thereof. Thus, it was confirmed that the decoy was introduced into the vascular adventitia and a part of the vascular media.

(Example 5: Effect of Decoy Nucleic Acid on Aortic Aneurysm Model Rats)

Aortic aneurysm model rats have been established (Holmes D. R., Petrinec D., Wester W., Thompson R. W., Reilly J. M., "Indomethacin prevents elastase-induced abdominal aortic aneurysms in the rat", J. Surg. Res., 1996, Jun; 63(1):305-9). This model can be produced by retaining elastase in the rat aorta under a pressure of 150 cm H_2O for 30 min.

As shown in Figure **10**, the cross section area of the aorta was significantly increased in the aortic aneurysm model rats which received the scrambled decoy. In contrast, such an increase was significantly suppressed after two weeks ("2W" on the horizontal axis in Figure **10**) and after three weeks ("3W") in the NF-KB and ets double decoy-administered group.

(Example 6: Effect on Eosinophilic Abnormality: NF-KB Decoy Therapy for Asthma Model Rat)

Next, asthma models were prepared and the effect of the decoy of the present invention was demonstrated. The asthma models were prepared basically in accordance with a technique described in Eur. J. Pharmacol., 1995, Dec 7; 293(4):401-12.

(Animals and Methods for Sensitization)

Brown Norway rats (8 to 10 weeks old; weight: 200 to 300 g) were obtained from Charles River Japan. The animals were treated in compliance with the spirit of animal protection in accordance with rules defined by Osaka University (Japan). 1 ml of a pyrogen-free physiological saline containing 4 mg of aluminum hydroxide and 1 mg of ovalbumin (OVA; Sigma, grade V) was subcutaneously injected in the neck to sensitize the rats. As an adjuvant, *Bordetella pertussis* vaccine (including 3×10^9 heat-inactivated bacteria) was intraperitoneally injected into the rats. The same solution except for ovalbumin was intraperitoneally injected to rats which were used as negative controls.

(Preparation of Sendai Virus Envelope Vector)

Sendai virus (HVJ) envelope vector (HVJ-E) was

prepared as follows. Briefly, virus suspension (1.0×10^4 hemagglutinating units (HAU)) was inactivated by UV irradiation (99 mJ/cm^2) and mixed with decoy oligonucleotide (200 μg) and 0.3% Triton-X, followed by centrifugation. Thereafter, the inactivated virus suspension was washed with 1 ml of balanced salt solution (BSS; 10 mM Tris-Cl, pH 7.5, 137 mM NaCl, and 5.4 mM KCl) to remove surfactant and unincorporated oligonucleotides. Following centrifugation, the envelope vector was suspended in an appropriate amount of phosphate buffered saline (PBS). The vector was stored at 4°C until use.

(Experimental Protocol)

12 days after sensitization, the rats were treated as follows. Using orotracheal instillation, (1) 0.5 ml of physiological saline (control); (2) 500 μg of naked decoy/0.5 ml; (3) 200 μg of decoy treated with HVJ-E 1.0×10^4 HAU/0.5 ml; (4) 500 μg of decoy treated with HVJ-E 2.5×10^4 HAU/0.5 ml HVJ-E; (5) HVJ-E 2.5×10^4 HAU/0.5 ml, were administered, respectively. On day 14, the rats were challenged with 5% aerosolized ovalbumin for 5 min using a nebulizer (PARI turbo). The airflow rate was 7 to 8 liters/min. As a result, airway irritation was induced. A flowchart of the experimental protocol is shown in Figure 11.

(Introduction of Decoy)

Decoys were administered into rats by nasal inhalation (using aerosol) or intubation (liquid, aerosol, respirator).

In the first experiment, decoys were administered with 2 mg×1 time, 1 mg×2 times, or 2 mg×2 times (aerosol),

or 0.5 mg × 1 time (liquid administration).

In the second experiment, decoys were prepared using HVJ-E as described above and were administered.

(Bronchial Alveolar Lavage (BAL))

BAL fluid was collected 24 hours after ovalbumin challenge. The rats were euthanized by an intraperitoneal injection of an overdose of sodium pentobarbital. BAL was performed using 5 ml of phosphate buffered saline (PBS; 137 mM NaCl, 10 mM sodium phosphate buffer pH 7.4, 2.7 mM KCl) 4 times. The total number of cells in the BAL fluid was determined using a hemacytocounter. Differential cell counts were performed by counting more than 300 cells with Diff-Quick stain (IBMC Inc., Chicago, IL: Catalog # K7124).

(Results)

Figure **12** shows the behavior of the percentage of eosinophils in BAL in the first experiment. It was found that ovalbumin challenge elicited a statistically significant increase in the numbers of eosinophils ($p<0.01$). After challenge, the aerosol treatment or the liquid treatment was performed. As a result, in either case, the increase of eosinophil was statistically significantly suppressed ($p=0.08$ for each). Therefore, it was demonstrated that the decoy of the present invention is effective for diseases causing eosinophilic abnormality, such as asthma and the like, irrespective of administration method.

Figure **13** shows an effect of the decoy of the present invention when Sendai virus was used as another administration method. After ovalbumin challenge,

treatment was performed using the above-described HVJ-E decoy prescription. As a result, the HVJ-E decoy had a significantly higher effect than the naked decoy. A HJV-E prescription having a 2.5-fold amount had a statistically significantly higher therapeutic effect (See Figure **14**). It was demonstrated that such an HJV-E effect was not attributed to the vector itself. Thus, in the present invention, it was demonstrated that the HVJ-E prescription has a higher therapeutic effect.

(Example 7: Treatment with Variant)

Next, decoy variants were used in aortic aneurysm and asthma model rats instead of the decoys used in the above-described examples. Whether or not the same effect can be obtained with decoy variants was confirmed. Here, as a variant, a consensus sequence of NF-κB, GGG(A/G)(C/A/T)T(T/C)(T/C)(C/A/T)C (SEQ ID NO. 13), was used. In the sequence listing submitted herewith, "r" represents A or G; "h" represents C, A, or T; and "y" represents T or C. Therefore, it was demonstrated that any sequence recognized by NF-κB can be used to treat and prevent diseases, disorders and/or conditions caused by expression of a gene controlled by NF-κB or ets, and diseases, disorders and/or conditions caused by abnormality of eosinophils.

The same experiments as in the above-described examples were conducted, except for use of different decoys. For either aortic aneurysm or asthma, it was demonstrated that therapeutic and prophylactic effects were exhibited. Therefore, the present invention is not limited to particular decoys. It was demonstrated that any decoy has the same therapeutic and prophylactic effects as long as the biological activity (i.e., activity to bind to a

transcriptional factor sequence) is maintained.

INDUSTRIAL APPLICABILITY

The present invention provides a pharmaceutical composition for treating diseases caused by expression of a gene controlled by NF-KB or ets, and diseases caused by abnormality of eosinophils. The present invention also provides a carrier used for the composition. The topical administration of the pharmaceutical composition is non-invasive. Therefore, the present invention provides a repeatable therapeutic method.

Various other modifications will be apparent to and can be readily made by those skilled in the art without departing from the scope and spirit of this invention. Accordingly, it is not intended that the scope of the claims appended hereto be limited to the description as set forth herein, but rather that the claims be broadly construed.